REMARKS/ARGUMENTS:

Claims 29-42, 55-62, 64-66, and 68-70 are pending in the application. Reexamination and reconsideration of the application, as amended, are respectfully requested.

Claim Rejection Under 35 U.S.C. § 112:

Claims 29-42, 55-62, 64-66 and 68-70 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification. In particular, the Examiner appears to believe that the claim language "without additional fixing steps" has no support in the specification. This rejection is respectfully traversed.

The specification provides a sufficient written description of the term "without additional fixing steps" although the terminology used in the claim differs from that in the specification. In its recent decision in All Dental Prodx, LLC v. Advantage Dental Products, Inc., 309 F.3d 774 (Fed. Cir., 2002), the Federal Circuit observed that "the failure of the specification to specifically mention a limitation that later appears in the claims is not a fatal one when one skilled in the art would recognize upon reading the specification that the new language reflects what the specification shows has been invented."

The present invention teaches immobilization of biopolymers by direct adsorption on a substrate. The specification defines "direct adsorption" as "adsorption ***without any chemical linkers***" (page 8, lines13-21). The specification emphasizes that the direct adsorption of the present invention results in biopolymers being ***immobilized on the substrate "in a stable way"*** (page 8, lines 13-21). Such stable immobilization does not require any additional treatment such as fixing steps.

As stated in the instant specification, conventionally, biopolymers are immobilized on substrates by ultraviolet cross-linking, chemical adhesion or covalent bonding (page 2, lines 19-21). Prior to the present invention, it was not known that biopolymers could be immobilized on substrates in a stable way by adsorption without further treatment. For example, Fareed (U.S. Patent No. 4,970,144), which was cited by the Examiner, discloses a two-step ELISA procedure, in which the proteins solutions are first dried in wells of 96-well dish and, then, they are fixed in the wells by methanol (column 14, lines 1-2).

In the present invention, the biopolymers that are adsorbed on the substrate by drying remain immobilized on the substrate during assays. Thus, additional immobilization (or "fixing") steps are not required. In fact, as evident from Examples 1 and 2 of the instant specification, the assay article is ***washed immediately after the drying*** step is completed to remove loosely bound biopolymers and used in the assays (page 16, lines 15-20, and page 17, lines 27-32). Thus, in the present invention, adsorption alone results in a stable immobilization of biopolymers and there is no need for additional fixing steps.

Furthermore, the term "fixing" is commonly used by those skilled in the art in the context of immobilization of biopolymers on a substrate (see, for example, several articles included herewith in Appendixes A-D). Based on the instant specification, particularly Examples 1 and 2, those skilled in the art would have recognized that the assay articles of the present invention are formed by adsorption without additional fixing steps. Those skilled in the art would have also understood that the phrase "biopolymer adsorbs and immobilizes on the modified surface without additional fixing steps" refers to the stable immobilization of biopolymers on the substrate by adsorption, which does not require additional treatment, such as fixing of biopolymers.

Therefore, based on the instant specification and in view of the common use of the term "fixing" in the art of biopolymer analysis, those skilled in the art would have understood that applicant, at the time the application was filed, had possession of the methods as claimed in claims 29 and 64.

Claims 29-42, 55-62, 64-66 and 68-70 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner rejected the claims because of the phrase "without additional fixing steps." This rejection is respectfully traversed.

As explained above, the phrase "without additional fixing steps" means that the adsorption of biomolecules on the substrate is sufficient for their ***stable immobilization*** without any additional fixing steps. As discussed above, the term is clear in view of the specification and the knowledge of those skilled in the art. Thus, the rejections under §112, first and second paragraphs, should be withdrawn.

Claim Rejection Under 35 U.S.C. § 102:

Claims 29-34, 36-38, 41-42 and 55-56 are rejected under 35 U.S.C. § 102(b) as being anticipated by Varma, U.S. Patent No. 5,622,826 (Varma). This rejection is respectfully traversed.

In the response to the Office Action dated March 17, 2003, applicant argued that Varma does not anticipate claim 29 for two reasons. ***First***, Varma does not teach a ***modified surface***, which is obtained by introducing a functionality selected from a group consisting of an amino group, a carboxyl group, a thiol group, and their derivatives. ***Second***, Varma does not teach the ***immobilization*** of a biopolymer on the modified surface ***by adsorption***. While the Examiner expressed her disagreement with the first argument raised by the applicant, she did not state

anything with respect to the second argument. However, the lack of a description of biopolymers immobilized by an adsorption in Varma, in itself, is sufficient to overcome the Examiner's rejection. Indeed, Varma does not teach the immobilization of a biopolymer on the modified surface by adsorption as required by step (c) of claim 29. Instead, Varma teaches coating substrates with a derivatizing agent comprising isocyanate or isothiocyanate (column 18, lines 16-23) and ***covalent binding*** of nucleic acids with reactive isocyanate or isothiocyanate moieties of the modified substrate (column 2, lines 55-56; column 6, lines 61-62; column 19, lines 29-36).

Additionally, applicant disagrees with the Examiner's interpretation of Varma with respect to the modification of the substrate's surface. The Examiner states on page 3 of the Office Action:

> ...Varma teaches that molecules bearing an amino group or functionality are immobilized on platinum surface by first reacting such surfaces with either an isocyanate or an isothiocyanate to produce immobilized reaction moieties on the surface (See column 2, lines 51-55). The instant claims are not limited to isocyanate or isothiocyanate which reacts on the surface to produce immobilized reactive moieties on the surface. Thus, Applicant's arguments filed on 6/6/2003 ... are not persuasive.

Applicant disagrees. Varma does not teach the modified surface of the present invention. As noted by the Examiner, claim 29 is not "limited to" isocyanate or isothiocyanate. Isocyanate or isothiocyanate groups are not recited in the claim. To the contrary, the ***closed transition "consisting of"*** limits the claim to surfaces modified with amino group, a carboxyl group, a thiol group, and their derivatives. Since isocyanate or isothiocyanate groups do not fall within any of the listed categories, they are ***excluded*** from the claim language.

Furthermore, the description of covalent binding of biopolymers on modified aminated polypropylene substrate, which has surface-bound isocyanate or

isothiocyanate groups (column 18, lines 24-25), does not anticipate claim 29. Step (d) of the instant claim 29 requires ***contacting biopolymer with the modified surface*** whereby the ***biopolymer adsorbs*** on the modified surface. The aminated polyprolylene substrate of Varma is ***modified with isocyanate or isothiocyanate*** to produce surface-bound isocyanate or isothiocyanate groups. In Varma, biopolymers chemically react with isocyanate or isothiocyanate. As a result, the biopolymers are immobilized on the substrate by ***chemical cross-linking*** and not by adsorption as in the present invention. Thus, Varma does not anticipate the modified surface of the present invention.

As explained in the previous response, Varma doesn't make the present invention obvious. It is an unexpected discovery of the present invention that substrates with the modified surfaces, such as plasma-aminated polypropylene and polysterene substrates, are capable of direct and ***stable adsorption*** of biopolymers without the need for chemical linkers and covalent binding. Consequently, the present invention provides a number of advantages over the conventional methods. The advantages include, for example, a simplification of the production of polypeptide arrays and a decrease in their manufacturing costs (page 5, lines 25-30).

Varma teaches a two-step immobilization of nucleic acids on aminated polypropylene substrates. The first step includes the ***further modification of aminated polypropylene by reaction with isocyanate or an isothiocyanate*** to obtain an activated surface. The second step is a ***covalent binding*** of a nucleic acid derivatized to contain an amino group with the reactive groups on the activated surface (column 3, lines 23-34; column 18, lines 7-25). These teachings of Varma demonstrate that, prior to the present invention, those skilled in the art did not expect that biopolymers could be immobilized directly on aminated polypropylene

substrates by adsorption and without the need for reactive groups such as isocyanates or isothiocyanates.

Thus, based on the teachings of Varma and without the hindsight of the present invention, those skilled in the art would not have realized that substrates with a modified surface having a functionality selected from a group consisting of an amino group, a carboxyl group, a thiol group, and their derivatives, can be used without further derivatization for the immobilization of biopolymers. Therefore, Varma does not anticipate or make present claim 29 obvious. Claims 30-34, 36-38, 41-42, and 55-56 depend from claim 29, directly or indirectly, and are patentable over Varma for at least the same reasons as claim 29.

Claims 64-66 and 68 are rejected under 35 U.S.C. § 102(b) as being anticipated by Fareed et al., U.S. Patent No. 4,970,144 (Fareed). This rejection is respectfully traversed.

In the response to the Office Action dated March 17, 2003, applicant argued that Fareed does not anticipate claim 29 for two reasons. ***First***, Fareed does not teach a ***modified surface***, which is obtained by introducing a functionality selected from a group consisting of an amino group, a carboxyl group, a thiol group, and their derivatives. ***Second***, Fareed does not teach immobilization by ***adsorption without additional fixing steps.***

The Examiner appears to believe that "[a]lthough Fareed et al. do not explicitly teach the functionality selected from a group consisting of an amino group, a carboxyl group, a thiol group, and their derivatives for immobilizing a probe or target polypeptide, the amide or ester linkage used by Fareed et al. produces an amino group or carboxyl group used in immobilization of biopolymer." Applicant disagrees. Although Fareed describes "***covalent bonding*** via an amide or ester linkage," he has no teaching whatsoever of modification of a substrate

surface with amino or carboxyl group to facilitate adsorption as the Examiner alleges (column 10, lines 24-29).

With respect to the applicant's argument that Fareed does not teach the language "without additional fixing steps," the Examiner appears to believe that the phrase "without additional fixing steps" is not defined in the specification. Accordingly, the Examiner interpreted this limitation as "any steps to immobilize biopolymer on the modified surface." Applicant disagrees.

As discussed in detail above, the limitation "without additional fixing steps" is supported by the specification as read in view of the knowledge of those skilled in the art. The limitation refers to the ***stable immobilization*** of biopolymers on the substrate by direct adsorption ***without chemical crosslinking***, which ***does not require additional treatment***, such as fixing of biopolymers. Fareed does not anticipate instant claim 64 because he does not teach "contacting either the probe or target polypeptide with the modified surface of the substrate and drying the substrate whereby either the probe or target polypeptide directly adsorbs and immobilizes on the modified surface without additional fixing steps."

Instead, as discussed in the previous response, Fareed teaches "typical ELISA assay" conducted with conventional microtiter dishes (column 11, lines 34-43). Such conventional microtiter plates do not have modified surfaces as defined in the present invention. Furthermore, in a "typical ELISA assay," a protein is not immobilized by drying as in the present invention, but rather chemically fixed onto the dish (see step 3 of the ELISA procedure in columns 13-14 of Fareed).

Fareed does not suggest immobilization of polypeptides by drying. At most, Fareed teaches a conventional ELISA assay with a two-step immobilization of protein. The first step involves an overnight drying of 50 µl of the protein solution in the wells of a conventional microplate and the second step involves the immobilization of protein by "filling the wells with absolute methanol to fix the

protein onto the dish" (column 14, lines 1-2). Based on such teaching, those skilled in the art would have not been motivated to modify the material of standard microplates to arrive at substrates with the modified surfaces of the present invention, much less to omit the protein-fixing step of the standard ELISA protocol without the hindsight of the present invention.

Therefore, Fareed does not teach or suggest the immobilization of polypeptides by drying on modified substrates and, thus, does not anticipate or make present claim 64 obvious. Claims 65-66 and 68 depend from claim 64, directly or indirectly, and are patentable over Fareed for at least the same reasons as claim 64.

Claim Rejection Under 35 U.S.C. § 103:

Claims 39-40 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Varma in view of U.S. Patent No. 6,197,501 (Cremer). This rejection is respectfully traversed.

As discussed above, claim 29 is patentable over Varma. Cremer does not remedy the defects of Varma and is not relied upon by the Examiner for such. The Examiner cites Cremer for the teaching of fluorescence labeling and using a CCD camera. Cremer does not teach or suggest adsorption of a biopolymer on a modified surface, which is obtained by introducing a functionality selected from a group consisting of an amino group, a carboxyl group, a thiol group, and their derivatives. Therefore, claim 29 is patentable over Varma in view of Cremer. Claims 39-40 depend from claim 29 and are patentable over the cited references for at least the same reasons as claim 29.

Claim 35 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Varma in view of U.S. Patent No. 6,013,789 (Rampal). This rejection is respectfully traversed.

As discussed above, claim 29 is patentable over Varma. Rampal does not remedy the defects of Varma and is not relied upon by the Examiner for such. The Examiner cites Rampal for the teaching of ELF. Therefore, claim 29 is patentable over Varma in view of Rampal. Claim 35 depends from claim 29 and is patentable over the cited references for at least the same reasons as claim 29.

Claims 57-62 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Varma and claims 68-70 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Fareed. This rejection is respectfully traversed.

As discussed above, independent claim 29 is patentable over Varma and independent claim 64 is patentable over Fareed. Claims 57-62 depend from claim 29 and are patentable over Varma for at least the same reasons as claim 29. Claims 68-70 depend from claim 64 and are patentable over Fareed for at least the same reasons as claim 64.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California telephone number (213) 337-6700 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-1314.

Respectfully submitted,
HOGAN & HARTSON L.L.P.

Dated: November 7, 2003

By: Barry Shuman
Barry Shuman
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Fixing Proteins on Electrophoresis Gels

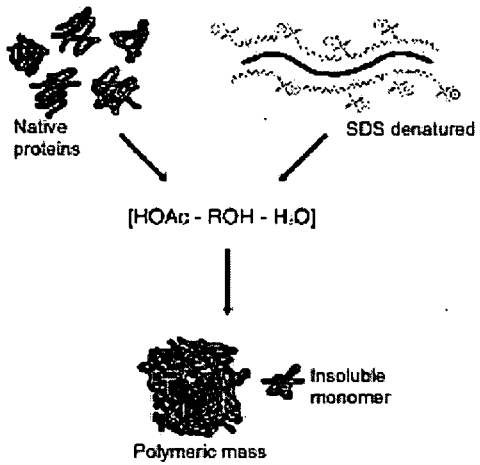


Figure 4.2.1a Fixing proteins with acetic acid and alcohol results in an uncoiling of the peptide chains to produce insoluble complexes and monomers.

Fixing (or fixation) is the process whereby proteins are denatured and precipitated in large insoluble aggregates within the gel matrix. Fixation accomplishes several goals. Primarily, fixation prevents the diffusion of proteins, thus keeping the protein bands sharp and resolved during the staining process. In addition, fixation removes gel buffer components, most importantly SDS, which may interfere in the staining process. In some cases, fixatives are used which modify the proteins to enhance the staining reaction.

An ideal fixative is fast, convenient and nonhazardous to use, and preserves the fine detail of the gel. It is important to be aware that fixing a protein within a gel drastically lowers the amount of protein which can be recovered from that gel after bands have been identified (see guide strip technique, Section 4.2.2). This is probably due to the trapping of gel matrix strands within the denatured protein complexes.

All fixatives operate by causing precipitation of the protein by converting it to an insoluble form. The most commonly used fixatives are solutions of short chain alcohols and acetic acid in water. The combination of low pH and high organic solvent content disrupts the hydrogen bonding which holds protein structures together, and exposes hydrophobic portions of the protein core. The result is an uncoiling of the peptide chain, followed by an essentially irreversible association between chains, producing a high molecular weight complex which is trapped inside the gel. This family of fixatives is cheap and relatively nonhazardous (depending on the alcohol used), and has the additional advantage that many stains are soluble in the fixative. This allows the combination of fixing and staining in one step. The only major drawback is that these solutions are only moderately denaturing, and may not fully fix small or unusually soluble proteins.

Stronger fixatives include trichloroacetic acid (12% in water), sulfosalicylic acid, or formaldehyde. TCA, sulfosalicylic acid and other strong acids act by protonating weak acids in the protein structure, disrupting the salt bridges and charge interactions required to maintain protein secondary structure. Aldehydes, such as formaldehyde and glutaraldehyde, react with amines on the surface of proteins, creating covalent cross links between protein molecules, resulting in a truly irreversible denaturation.

Appendix A

h ee f g c e f gh

Fixing Difficult Proteins

Small or unusually soluble proteins may not be sufficiently fixed by the above protocol. As these proteins diffuse through and out of the gel, smeared bands and loss of sensitivity may result. Prefixing of the gel in 12% trichloroacetic acid for 1-3 hours at room temperature prior to fixing by the above protocol will generally improve the fixing, and hence the staining of such proteins.

In certain cases, where proteins are heavily glycosylated or strongly basic, acid based fixatives may be ineffective. Small peptides may also be resistant even to strong acid fixatives. In such cases an effective alternative to acid precipitation is covalent cross-linking of the proteins with formaldehyde or glutaraldehyde. Formaldehyde fixation may be accomplished in a solution of 25% Ethanol, 15% Formalin (Formalin is 35% formaldehyde), 60% water. Gels are submerged in this solution for 1 hour, and may then be stained with or without subsequent alcohol/acetic acid fixation. Glutaraldehyde is generally used as a fixative in Silver Staining. Gels are soaked in 10% aqueous glutaraldehyde for 30 minutes, then washed for 2 x 20 minutes with water before staining. This denatures the proteins and fixes them in the gel; it also puts reactive aldehyde groups on the surface of the proteins, which enhance the silver stain reaction.

h ee f g c e f gh

Technical Library

Optimization of Southern Blotting Performance on Positively Charged Nylon Membranes

Protocol
PC032

Michael A. Mansfield and Constance G. MacDonald

Abstract

Charged nylon membranes are commonly used as a support for hybridization of nucleic acids, but the parameters for optimal blotting performance have been poorly understood. In this poster, we describe optimization of DNA transfer and UV cross-linking conditions on Immobilon-Ny+. Remarkable enhancement of sensitivity and re-probing ability is thus realized. In terms of signal strength, alkaline transfer is shown to be inferior to standard transfer in 20X SSC, although either method can be utilized. UV cross-linking is the best method for **fixing DNA** to nylon membranes as this process results in covalent attachment of the DNA to the nylon. In this study, the performance of Immobilon-Ny+ in Southern blotting was analyzed as a function of transfer conditions and optimization of UV cross-linking. In addition sensitivity and reprobing characteristics were compared to other commercially available charged nylon membranes.

Materials and Methods

Electrophoresis and Capillary Blotting.

Lambda Hind III fragments were resolved electrophoretically on agarose and blotted to Immobilon-Ny+ (positively charged nylon membrane, 0.45 µm, Millipore) overnight by capillary transfer. The blots were dried prior to UV fixation.

UV Fixation of DNA. Transfer, cross-linking and stripping protocols are described in more detail in Millipore Technical Notes TN054, TN055 and TN056. Transferred DNA was UV cross-linked to the membrane at 254 nm using a Stratalinker (Stratagene Cloning Systems, La Jolla, California, USA) after drying the membranes.

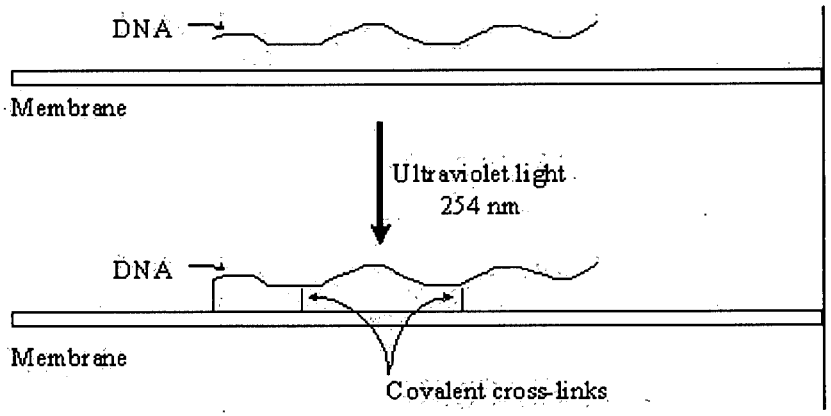
Hybridization. Pre-hybridization was done for 2 h at 68°C; then hybridization solution containing 32P-labeled probe (Hind III DNA fragments) was added and incubated for 16 to 20 h at 68°C.

Imaging. Radioactivity on the membranes was visualized by phosphor imaging on a Storm 840 Phosphor Imaging System (Molecular Dynamics, Sunnyvale, California, USA); then quantified using ImageQuant analysis software.

Appendix B

h e ch c che c c g e c b c f c f g

UV Cross-linking of DNA on Immobilon-Ny+



Comparison of SSC and Alkaline Transfer on Immobilon-Ny+

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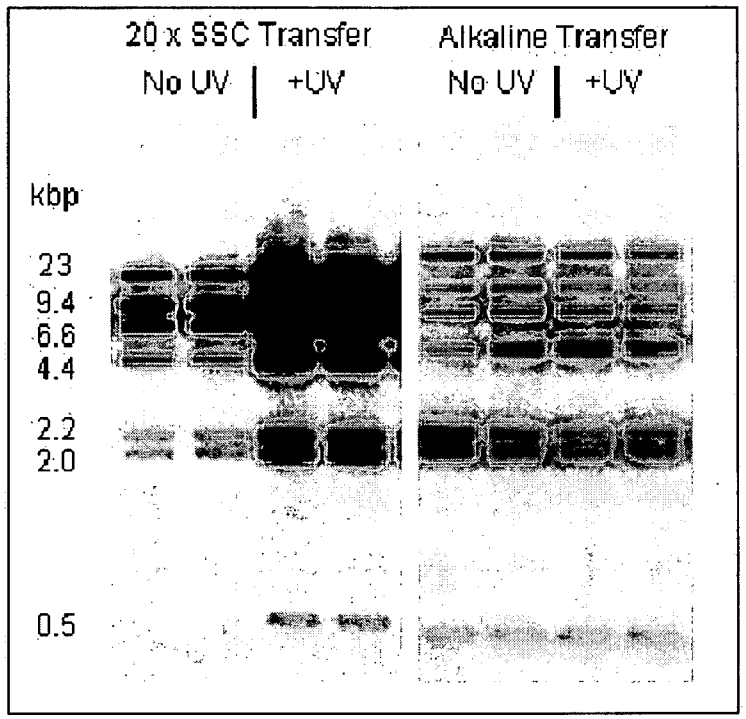

Relationship Between DNA Retention and Hybridization Signal Intensity

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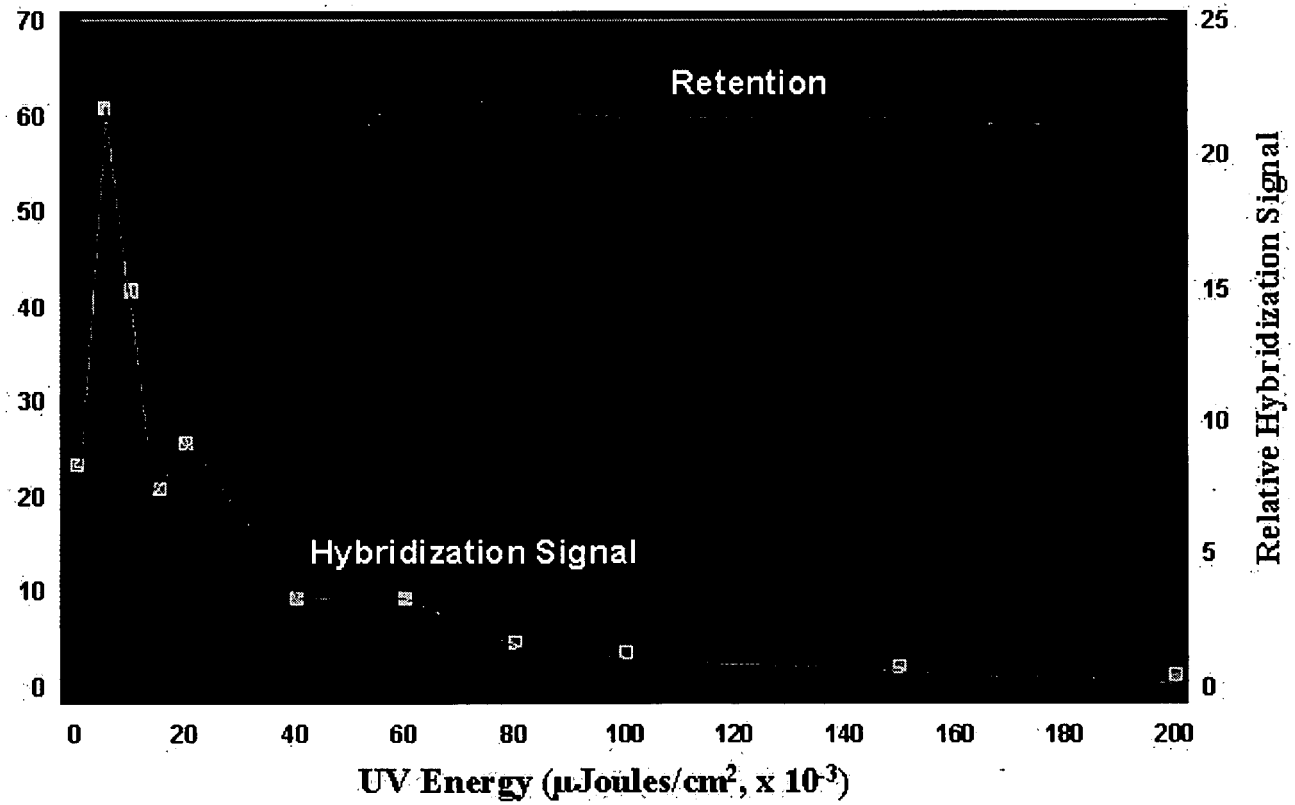

Effect of UV Energy on Hybridization Signal Intensity

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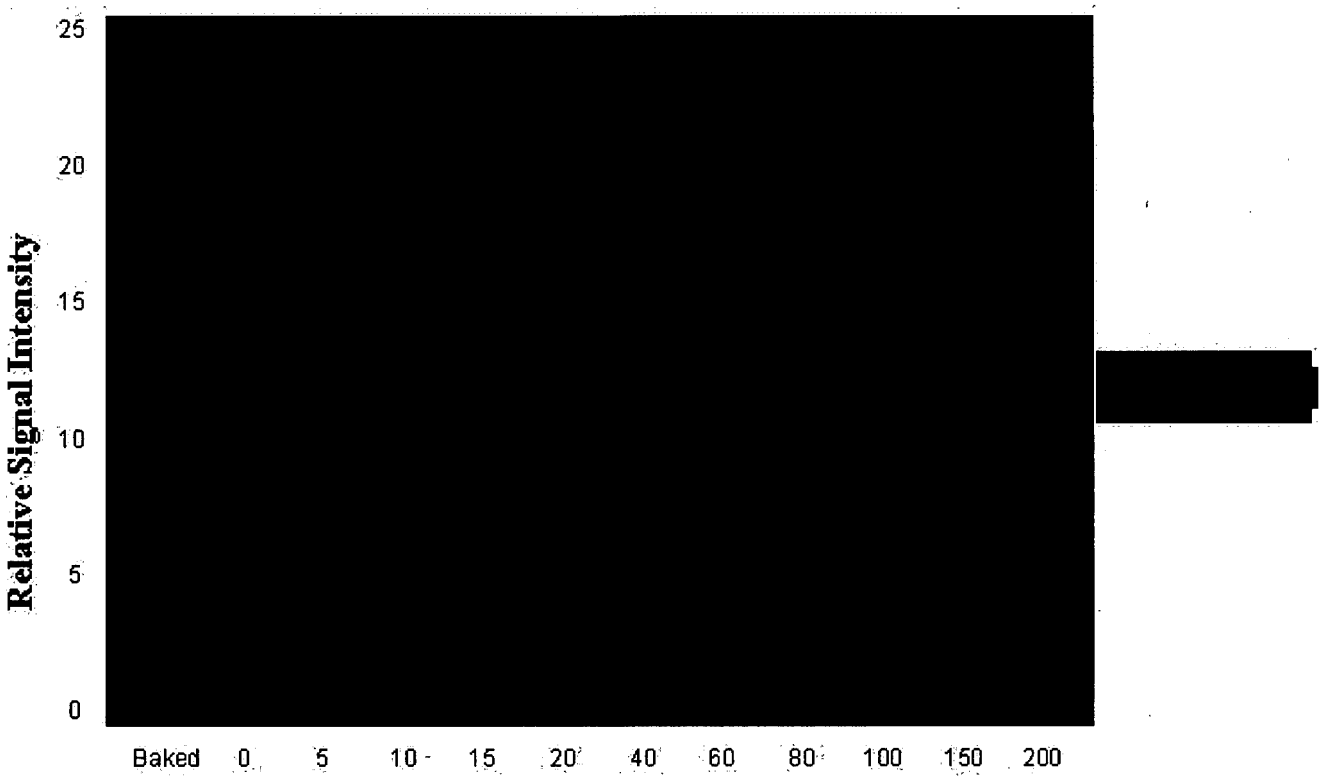

Change in Hybridization Signal with Multiple Reprobes

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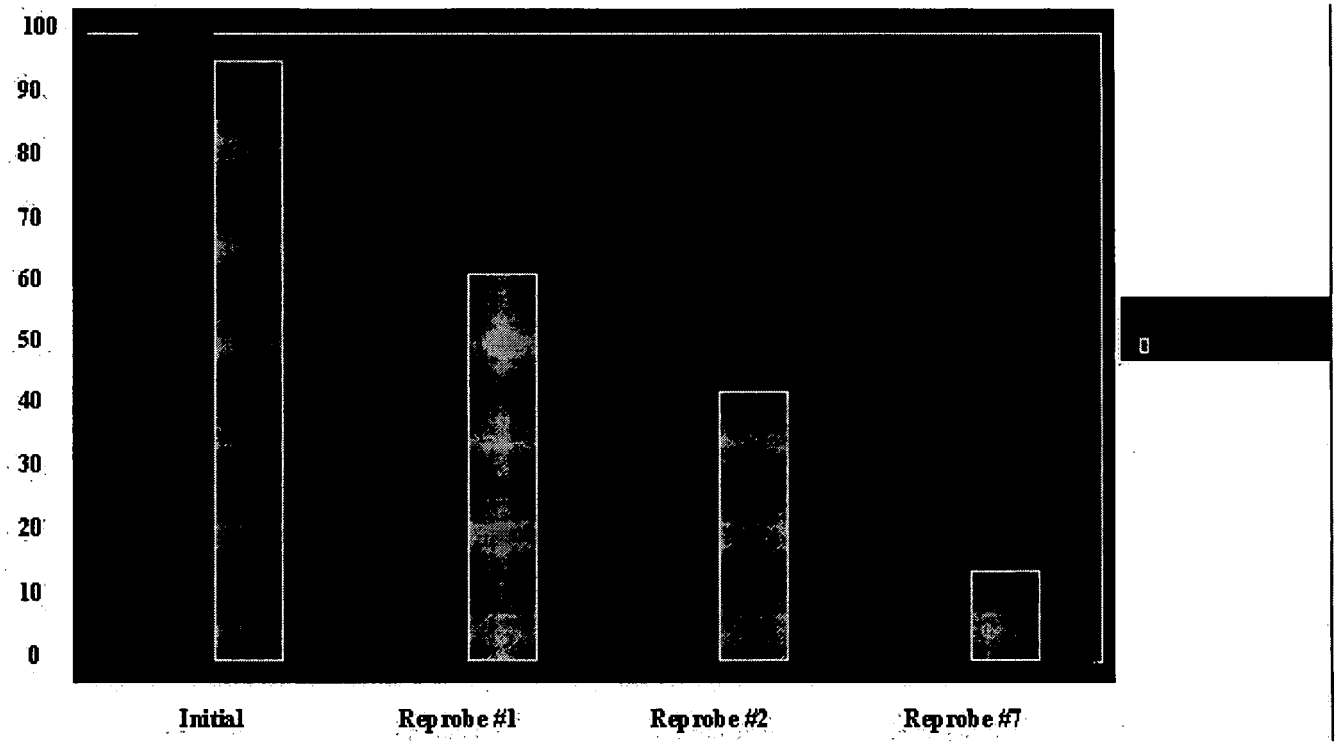

Reprobing of Immobilon-Ny+, Round 13

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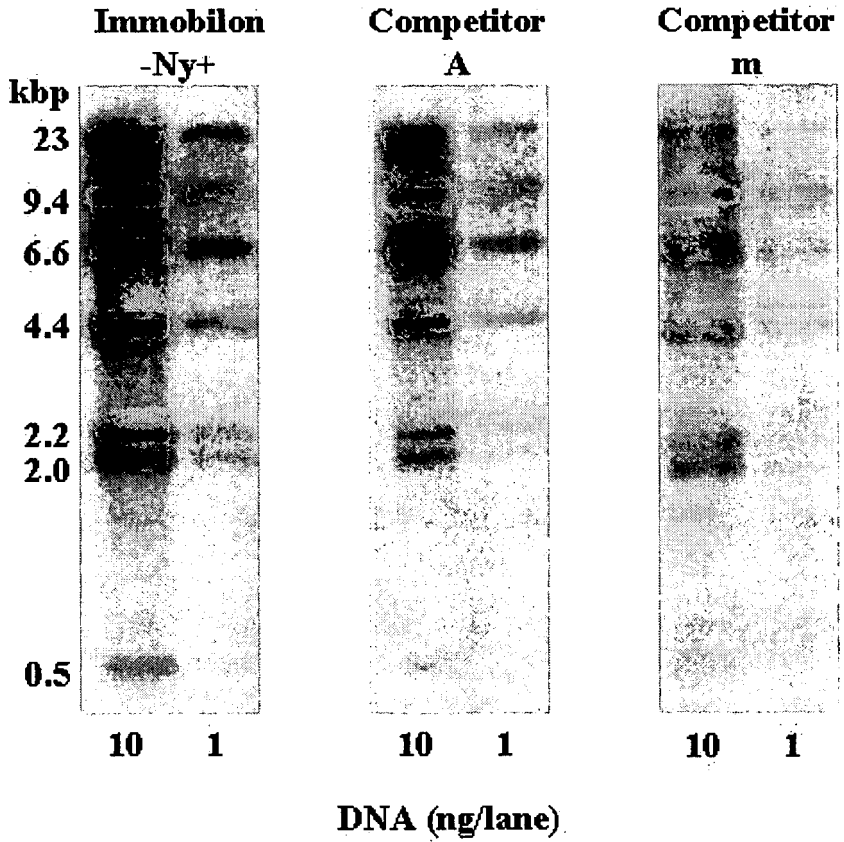

Conclusions

The efficiency of all nucleic acid hybridization assays on membranes is dependent on four major factors: elution of target DNA from the gel during transfer, binding of target DNA to the membrane during blotting, retention of target DNA during hybridization and stringency washes, and accessibility of the target DNA to the probe molecule. Without guidelines for fixation of target DNA to blotting membranes, DNA can be under-cross-linked and the target lost, or, over-cross-linked and the target rendered inaccessible to the probe. For optimal blotting performance with Immobilon-Ny+, the recommendations below should be followed:

The DNA must be applied to the membrane in single-stranded form (i.e., denatured).

h e ch c che c c g e c b c f c f g

Transfer is best done using 20 x SSC. Alkaline transfer is inferior, but still an option.

UV cross-linking is the preferred method for DNA fixation. We recommend 5,000 µJoules/cm2 for optimal hybridization sensitivity as higher energy levels cause a rapid loss of signal.

GO ▸ | Product Information

Catalog Specifications

| English | Français | Italiano | Deutsch | Español | Português | 日本語

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h e ch c che c c g e c b c f c f g

Appendix C

UVP

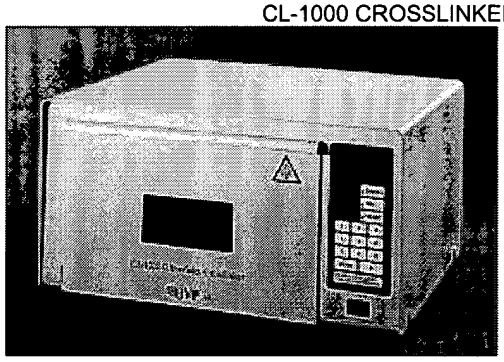
UVP, Inc. 2066 W. 11th Street, Upland, CA 91786
Tel: (800) 452-3197 or (909) 946-3197
Fax: (909) 946-3597
E-Mail: uvp@uvp.com

Application Bulletin
Bull tin UVP-AB-114
Ultra-Viol t Products Limited, Science Park,
Cambridge, England, CB4 4FH
Tel: +44(0)1223-420022 Fax: +44(0)1223-420561
E-Mail: uvp@uvp.co.uk

USES OF THE CL-1000 UV CROSSLINKER IN THE LABORATORY

The CL-1000 UV Crosslinker is a multi-purpose ultraviolet exposure instrument for use in the laboratory. Utilizing a 254nm shortwave ultraviolet (UV) radiation, the Crosslinker has the ability to perform a wide variety of applications.

CL-1000 CROSSLINKER



INTRODUCTION

Ultraviolet radiation is a fast, easy, and effective method to fix nucleic acids to nitrocellulose, nylon, and nylon-reinforced membranes after Northern, Southern, slot or dot blotting. Ultraviolet radiation catalyzes the covalent attachment of nucleic acids to these membranes by activating interactions between thymines or uracils with the amine groups on the membrane matrix [1]. The result is higher resolution and sensitivity of subsequent hybridization analysis. The entire fixing process performed on the Crosslinker is around two to ten minutes, compared to the two hour period required for fixing by the baking method.

The Crosslinker is not limited to just fixing nucleic acid. The versatile wavelength is effective in many applications, compatible with most molecular biology experiments. CL-1000 UV Crosslinker can be used to nick ethidium bromide stained DNA in agarose gels, a step in alternating contour-clamped homogeneous electric field gel electrophoresis (CHEF) [2]. CHEF is a type of pulsed field gel electrophoresis, when used with the CL-1000 UV Crosslinker, can resolve DNA fragments greater than five megabases with clarity and ease.

UV irradiation of DNA provides an easy way to control the extent of restriction endonuclease digestion due to the fact that UV radiation dimerizes neighboring thymidines (TT, TTT, etc.). The restriction enzymes can not recognize and cleave the DNA if the thymidines within their restriction sites were dimerized [3]. The Crosslinker allows a greater control over partial digestions.

The Crosslinker offers a simple method to test for *recA* mutations. *RecA+* strains repair UV-induced damages and grow normally whereas mutations in *recA* prevent the cells to grow because of inability to repair damages [4]. By irradiating a strain with an unknown genotype, a mutation can be easily detect due to the properties mentioned above.

Ultraviolet radiation is often used in sterilization. The Crosslinker is effective in killing bacteria cultures, viruses, bacteriophages, and small organisms on surfaces [5]. It is an efficient alternative to the traditional heat germicide.

UV radiation can solve the problem of PCR (polymerase chain reaction) contamination. UV irradiated fragments form pyrimidine dimers which function as termination sites [6]. The formation of these termination sites eliminates most contamination caused by the reagents from the previous amplified material.

MATERIALS AND METHODS

A. FIXING NUCLEIC ACIDS ON TO MEMBRANES

Run agarose gel electrophoresis [7]. Transfer nucleic acids on to the membrane by capillary or other transfer methods [8]. Lay the membrane, nucleic acid side up, on a piece of tin foil. Do not cover. Turn the Crosslinker on. Press one of the

auto settings: UV energy exposure or time. Remove the membrane when the Crosslinker beeps five times. Continue with hybridization [9, 10].

B. NICKING ETHIDIUM BROMIDE STAINED DNA IN AGAROSE GELS

Prepare DNA samples. Run CHEF gel electrophoresis using a hexagonal array of electrodes. After electrophoresis, the gel is stained with ethidium bromide (0.5 µg/ml) and irradiated by the Crosslinker for about one minute [2]. The gel is then subjected to hybridization [10].

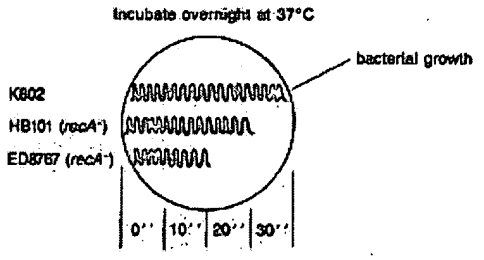
C. GENE MAPPING FOR CREATING CLEAVAGE-INHIBITING THYMINE DIMERS

Prepare DNA samples. Add 0.1-1 µg of DNA and 20 µl of restriction buffer. The restriction buffer must contain MgCl2. In the experiment conducted by Whittaker it was added into the mixture when the appropriate oligo was end-labeled. This mixture is irradiated for up to 60 minutes [3]. Then the mixture can be analyzed by agarose gel electrophoresis [7]. Longer irradiation time equals less cleavage by the restriction enzymes.

D. TESTING recA FUNCTION

Streak the strain being tested along with *recA+* strain on a petri dish. Us a piece of cardboard to cover about three quarters of each streak, expose the remaining portion to the Crosslinker for about ten seconds. Move the cardboard so half of the dish is covered. Expose the dish to the Crosslinker for ten seconds. Then move the cardboard so three forth of the petri dish is exposed, place it in the Crosslinker for ten seconds. Incubate the dish at 37°C overnight [4]. The *recA-* strain should be shriveled and shorter than the wild type strain (fig. 1).

fig.1



E. ULTRAVIOLET STERILIZATION

Place the surfaces to be sterilized in to the Crosslinker for a set amount of time. Consult the Bacteria Destruction Chart on the UVP Internet home page for suggested time. Note: the Crosslinker cannot sterilize liquid nor solids.

F. ELIMINATION OF PCR CONTAMINATION

Irradiate the target DNA with the Crosslinker, five minutes is sufficient. For best results, the photoinduced defects should be in the sequence region bounded by the 3' ends of the PCR primers [11]. Add the PCR reaction components. Perform amplification as practiced [12].

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ETC

Elektrophorese-Technik

Strategy of Optimization for Silver Staining

Okt 27, 20

Droplet-Test | DNA-Silver | IEF-Silver | SDS-Silver

Home
Ready To Use
CleanGels
MiniCleanGels
Buffers
Hardware
Clean-Kits
Consumables
Applications
ETC SilverStain
SDS-Elpho
Fluorescence
IEF

General:

The pinciple of silver staining is the following:

In the gel the soluble, non visible Ag^+ -ions are reduced
to the metallic, black, and visible Ag^0.

The Ag+ donor is: $AgNO_3$

At the same time, the redox potential has to be chosen in that way, that the Ag+ ion, which is complexed in the polyacrylamide gel, can only react in the presence of an additional compound: a biomolecule like a protein or DNA.

The reducing solution: 0.037 % formaldehyde, and pH 12 due to 2.5 % sodium carbonate

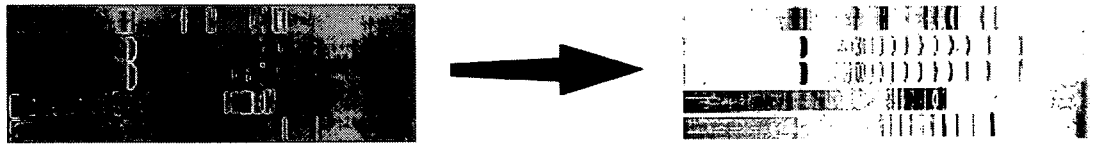
To avoid re-desolving of precipitating Ag+-ions (brown, visible), such as AgCl, use:

Ag+ -complexing compound: 0.002 % sodium thiosulfate (only DNA silver-staining)

Appendix D

h e ch c che e f e ce h c h e h f g ge h

Native-Elpho
DirectBlot
ReAmp
SSCP
Sortex
Download
ImageTransfer
Seminar
Contact



Solutions and Procedure (General, DNA-Silver-Staining)

A Fixing and Washing

At the beginning, the **gels** have to be fixed and washed. Different recipes for proteins and DNA.

***Fixing DNA-gels**: 15 % ethanol / 5 acetic acid with the gel swimming on the liquid, gel side looking downwa min at room temperature, 20 min when preheated to 50°C Washing with Bidest, gel swimming on the surface looking downward: 3 times 13 min at room temperature, 3 times 5 min when preheated to 50°C*
Newest recipe: Fixing with 0.6% Benzenesulfonic acid (free acid) [Merck 468] in 24% Ethanol

B The Silver Solution (prepare freshly)

0.1% $AgNO_3$ [Merck 1512] brings the Ag+ ions into the gel; stock solution: 2% > 10 ml silver solution + 200 formaldehyde (37%) [Merck 4003] per 200 mL Formaldehyd is the reducing reagent, but does not yet work, because the pH value is slightly acidic.

During the silvering, the gel lays at the bottom of the tray, gel side looks upward! 45 min at room temperatur min when preheated to 40°C

C The Developer (prepare freshly):

2.5 % Na_2CO_3 [Merck 6392] shifts the pH to 12, fast start, because formaldehyde is already present (silver solution!), stock solution: 10%. 0.037% formaldehyde reduces - together with the biomolecules - the Ag+ -io 0.002% sodium thiosulfate [Merck 6516] complexes precipitating Ag+ -ions (brown background!); recommen stock solution: 2%.>50 ml Na_2CO_3 + 200 µ l Formaldehyd + 150-200 µl Na-thiosulfate per 200 ml.

During the developing step, the gel lays at the bottom of the tray, gel side looks upward!3-6 min at room

h e ch c che e f e ce h c h e h f g ge h :

temperature, dont heat! Best results when precooled to 8°C!

Testing and optimization of the 2 imortant solutions: The Droplet-Test (Button below)

D Stopping, Preserving and Drying

At the end, the **gels** have to be desilvered, preserved and dried.

.This can be done in one solution: 2% Glycin + 0.5 EDTA-Na. 20 min at room temperature. Air dry overnight

h e ch c che e f e ce h c h e h f g ge h :

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